

EP02.08: From antibiotic to anticancer: repurposing doxycycline as a co-therapy with targeted lung cancer drugs

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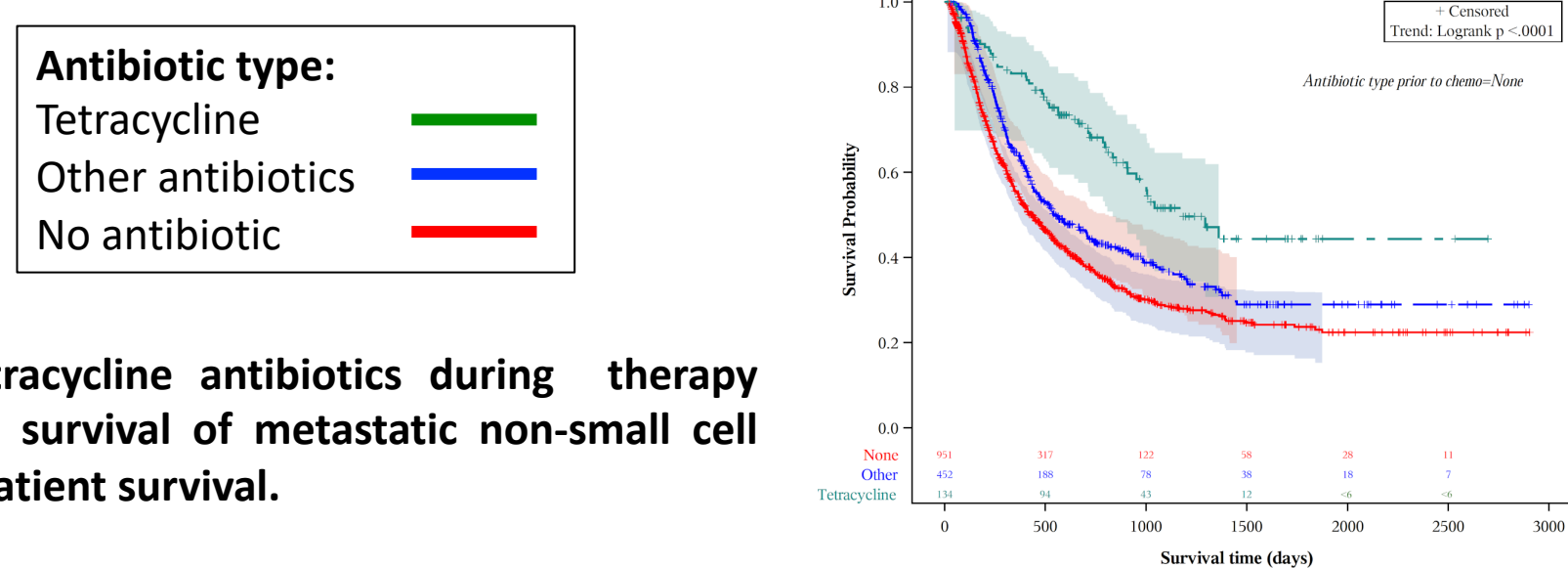
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INTRODUCTION

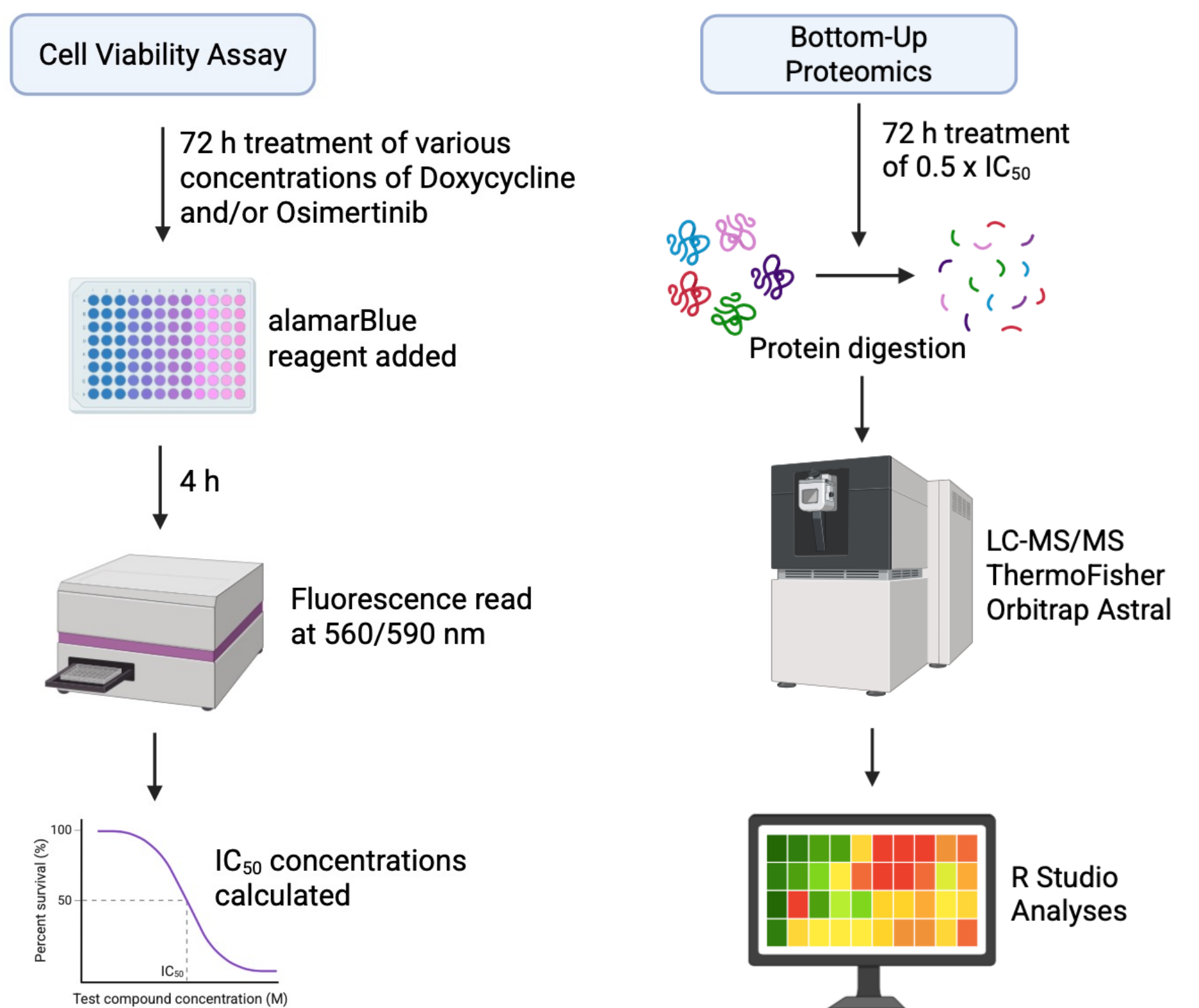
Continued advances in lung cancer therapy have improved outcomes, yet a critical need remains to develop additional treatment options. Currently unpublished epidemiological findings from Australia indicate that concurrent exposure to the antibiotic doxycycline was associated with a 30% increase in 3-year survival in people with metastatic non-small cell lung cancer undergoing systemic therapy (2013-2019)(Figure 1). These findings align with earlier reports suggesting that the use of tetracycline antibiotics during therapy has been previously associated with improved lung cancer survival^{1,2}. Doxycycline has been linked to multiple anticancer mechanisms, including the disruption of mitochondrial biogenesis³, enhancement of anti-tumour immunity⁴, and inhibition of matrix metalloproteinases⁵; therefore, it is a promising therapeutic avenue to enhance lung cancer treatment. Given the introduction of new targeted agents since 2019, the most effective drug combinations with doxycycline have yet to be established.



AIMS

- Identify the most beneficial combinations of doxycycline and epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) in cultured lung cancer cells.
- Elucidate the underlying molecular mechanisms responsible for this effect.

METHODS



RESULTS

HCC827Luc – EGFR Exon 19 Deletion

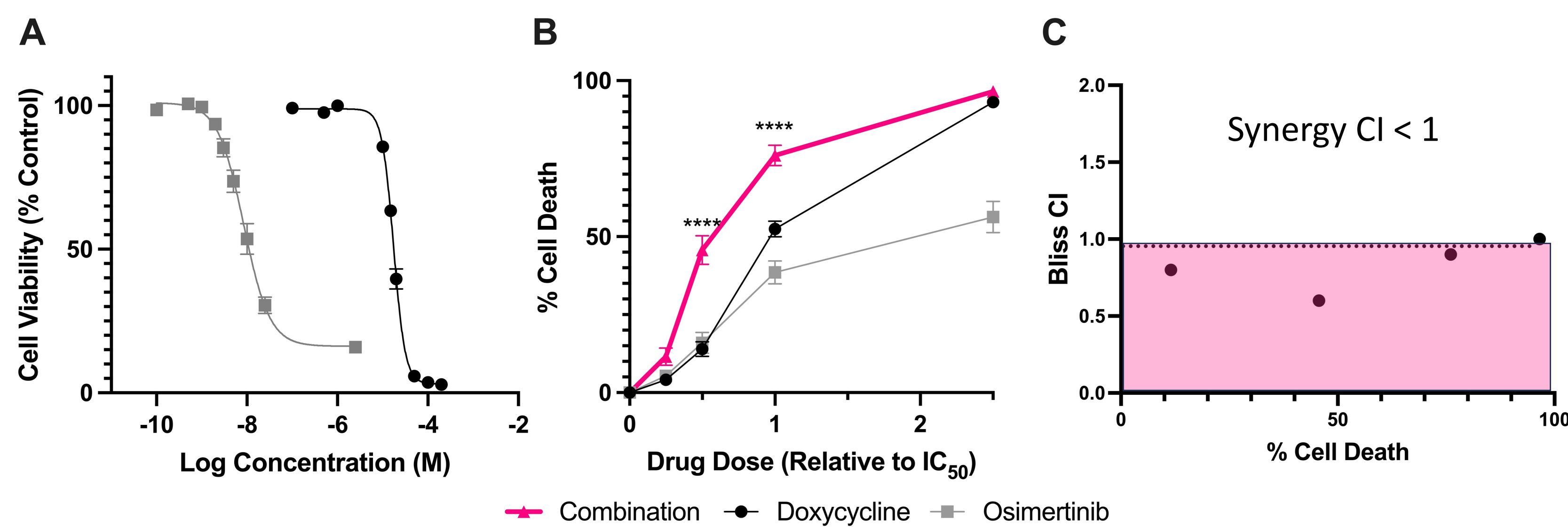


Figure 2. The effect of doxycycline, osimertinib, and their combination on cell viability of HCC827Luc cells. (A) Effect of doxycycline and osimertinib as monotherapies on cell viability. IC₅₀ values were determined using non-linear regression analysis. (B) Effect of treatment on cell death. Data points expressed as mean ± SEM and analysed using a one-way ANOVA followed by a Bonferroni post hoc test. ****P < 0.0001. Synergy was assessed using the Bliss method to calculate combination index (CI) values. For all data n = 5 independent experiments performed in technical triplicate.

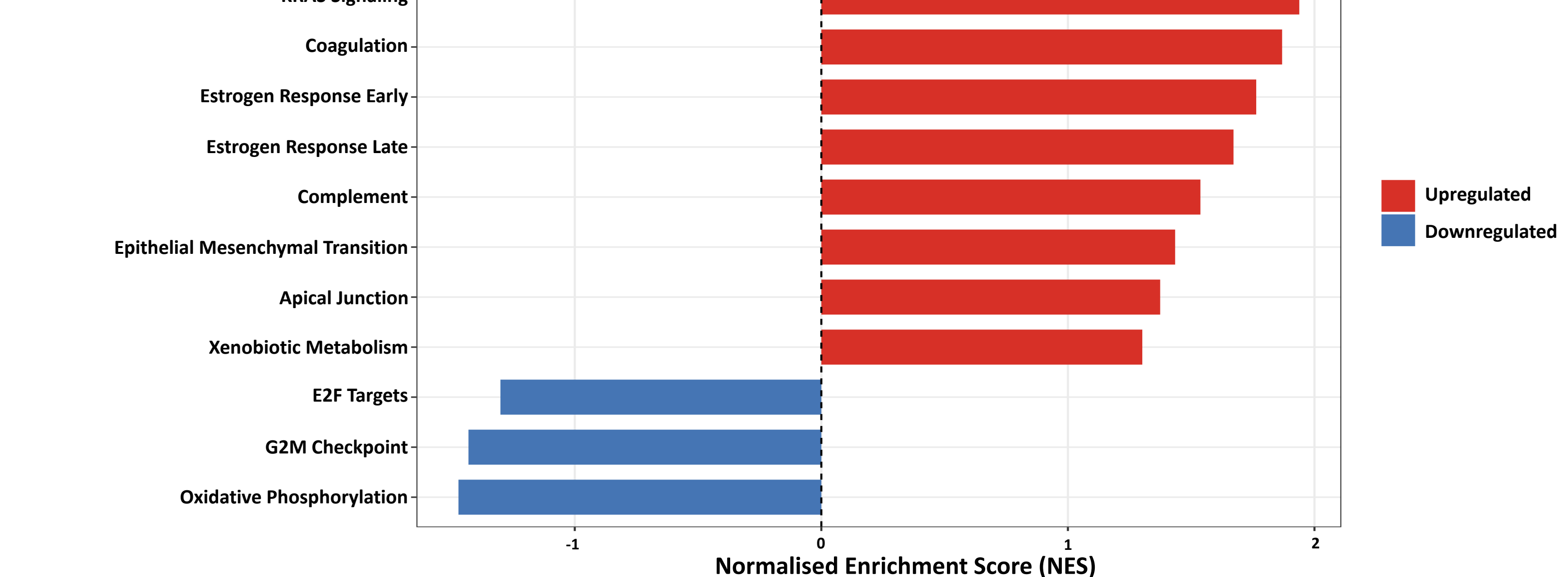
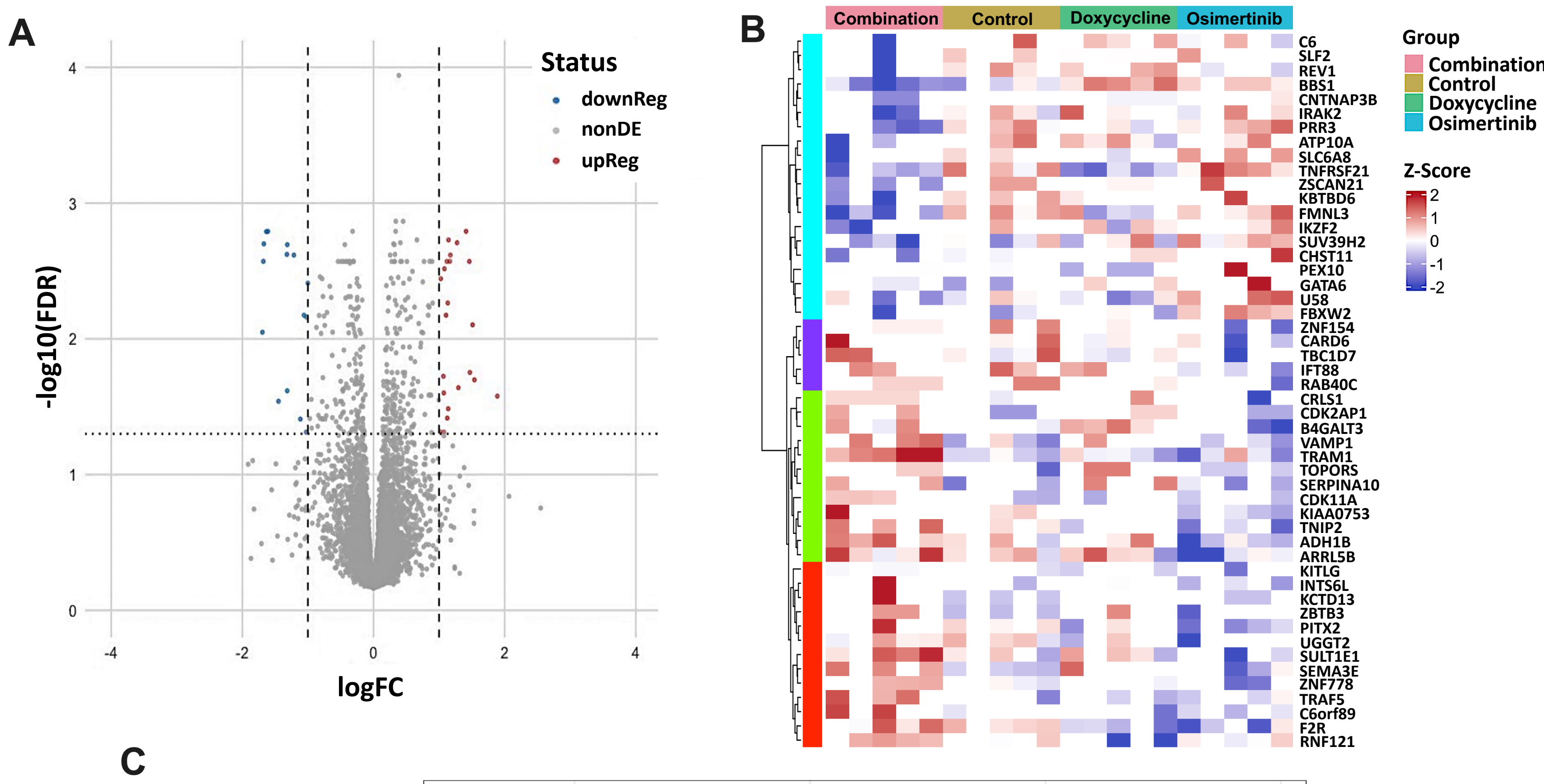


Figure 5. Proteomic quantification and pathway analyses of HCC827Luc lung cancer cells following 72-hour treatment with doxycycline + osimertinib or osimertinib alone (A) Volcano plot identified 22 upregulated and 15 downregulated proteins. (B) Heatmap of the top 50 differentially abundant proteins between the combination and osimertinib treatment. (C) GSEA Hallmark pathway analyses of the significantly enriched up and down regulated pathways.

H1975 – EGFR T790M/L858R Mutant

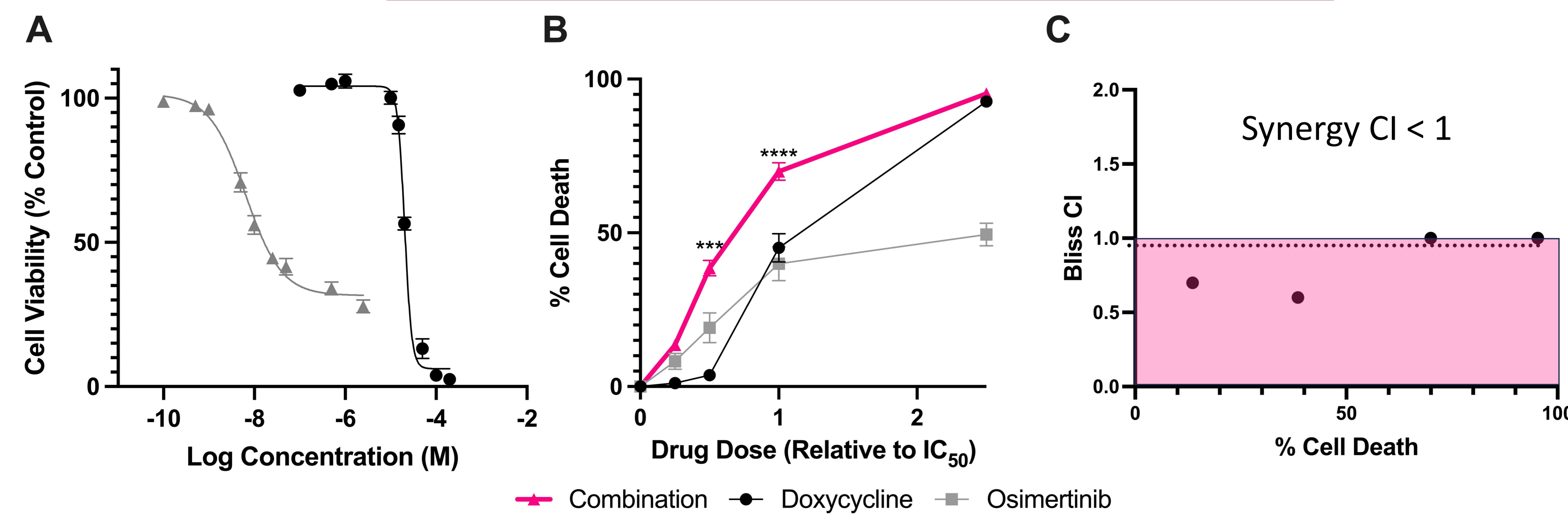


Figure 4. The effect of doxycycline, osimertinib, and their combination on cell viability of H1975 cells. (A) Effect of doxycycline and osimertinib as monotherapies on cell viability. IC₅₀ values were determined using non-linear regression analysis. (B) Effect of treatment on cell death. Data points expressed as mean ± SEM and analysed using a one-way ANOVA followed by a Bonferroni post hoc test. ***P < 0.001, ****P < 0.0001. Synergy was assessed using the Bliss method to calculate combination index (CI) values. For all data n = 5 independent experiments performed in technical triplicate.

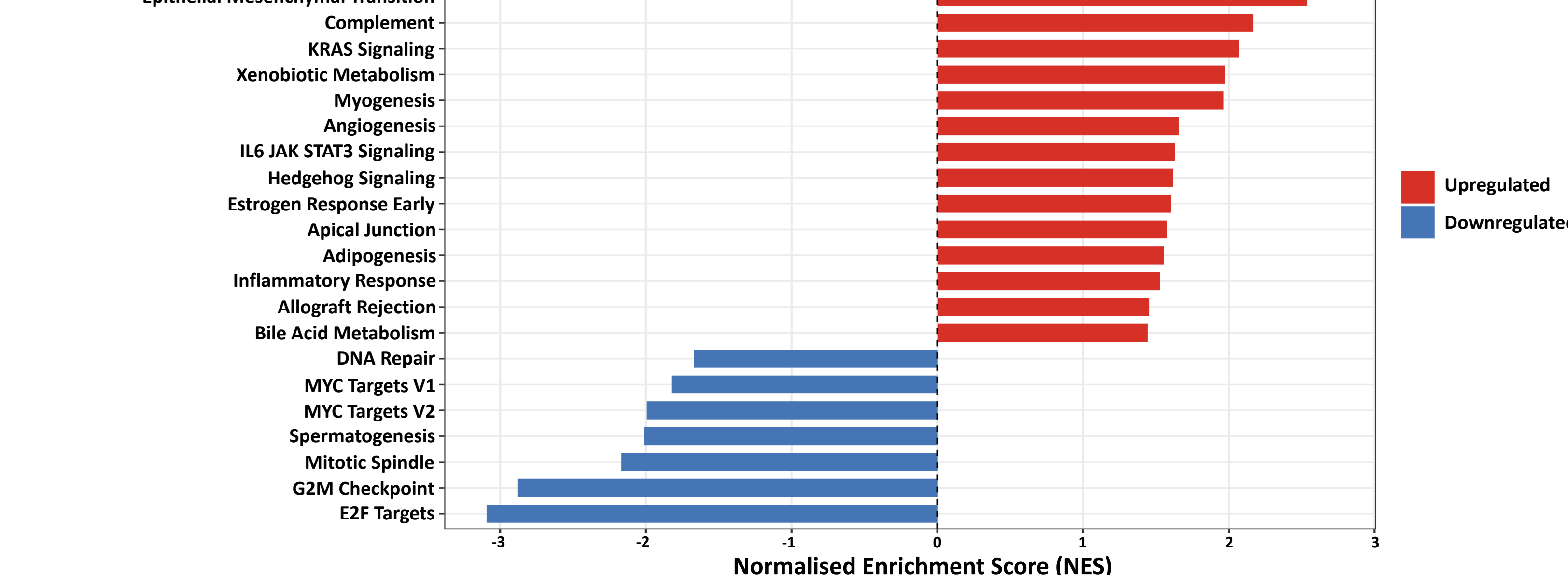
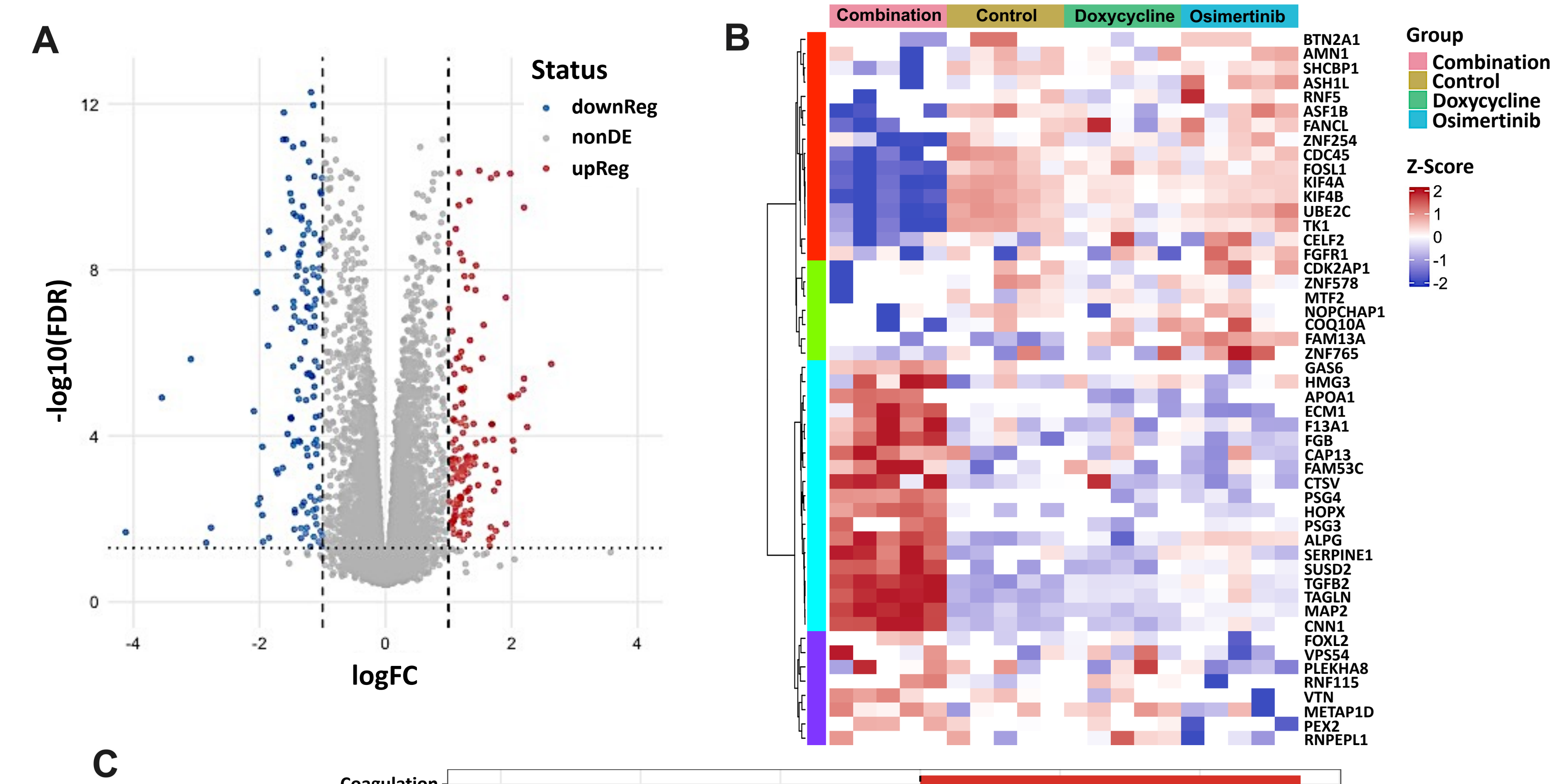


Figure 5. Proteomic quantification and pathway analyses of H1975 lung cancer cells following 72-hour treatment with doxycycline + osimertinib or osimertinib alone (A) Volcano plot identified 143 upregulated and 133 downregulated proteins. (B) Heatmap of the top 50 differentially abundant proteins between the combination and osimertinib treatment. (C) GSEA Hallmark pathway analyses of the top significantly enriched up and down regulated pathways.

DISCUSSION

- Doxycycline enhances the efficacy of EGFR TKIs with synergy at physiologically relevant concentrations, suggesting a beneficial molecular interaction in cultured lung cancer cells.
- Proteomic analyses of the addition of doxycycline to osimertinib revealed:

In both cell lines:

- ↓ cell proliferation
- ↓ cell cycle progression
- ↓ oxidative phosphorylation
- ↑ ‘adaptive’ survival with metabolic changes

H1975 cells only:

- ↓ DNA replication
- ↓ DNA repair pathways
- ↑ extracellular matrix remodelling with epithelial to mesenchymal transition

HCC827Luc cells only:

- ↓ cell migration and metastasis
- ↑ epigenetic reprogramming
- ↑ protein homeostasis (proteostasis) stress adaptation

- Future studies will further examine the synergistic combinations and molecular mechanisms in an *in vivo* model, specifically investigating repeated dosing and the role of the immune system.

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